Supporting Information

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SI Materials and Methods

Antibodies. The phospho-dAkt and total dAkt antibodies were purchased from Cell Signaling Technology, Inc., the phospho-JNK antibody was purchased from Promega, and the total JNK antibody was purchased from Santa Cruz Biotechnology. The E7 beta-tubulin antibody generated by Michael Klymkowsky was obtained from the Developmental Studies Hybridoma Bank. HRP-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology.

Immunoblot Analysis. Fat bodies from 4- to 5-day-old female flies were dissected in 1× PBS and homogenized in buffer containing 2% SDS, 60 mM Tris-HCl, pH 6.8, 1× protease inhibitor mixture (Roche Diagnostics), and 1× phosphatase inhibitor mixture 1 (Sigma). The lysates were then centrifuged for 15 min at 13,000 rpm at 4 °C. Protein concentrations were determined using the BCA Protein Assay Kit (Pierce). Equal amounts of protein (20–30 μg) were separated by SDS/PAGE followed by transfer to nitrocellulose membranes (Whatman). Detection was performed using ECL reagents (Amersham Pharmacia Biotech) and quantification was performed using NIH Image software.

Protein and Triglyceride Assays. Single adults or larvae were homogenized in buffer containing 140 mM NaCl, 50 mM Tris-HCL, pH 7.4, 0.1% Triton-X-100, and 1× protease inhibitor mixture (Roche Diagnostics). Protein concentrations were measured using the BCA Protein Assay Kit (Pierce) and triglyceride concentrations were measured using the Triglyceride Liquicolor Kit (Stanbio Laboratory) according to the manufacturer's instructions.

dFoxo Quantitation. The amounts of dFoxo localized to the nucleus and cytoplasm of fat body cells were calculated using Image J. Following background correction, a rectangular area was selected in each DAPI image, and the selected area was copied to each corresponding dFoxo image. DAPI selections were subjected to thresholding to allow selection of nuclei. The selected nuclear areas were copied to the corresponding dFoxo image, and mean gray values were measured in the nuclear and cytoplasmic areas of each dFoxo image. Nuclear to cytoplasmic ratios of dFoxo for each genotype were calculated using these mean gray values.

RNA Isolation and Quantitative PCR. Total RNA was isolated by a hybrid TRIzol (Invitrogen)/RNeasy (Qiagen) protocol. Briefly, animals were flash frozen and then homogenized in TRIzol reagent. RNA was chloroform-extracted and one volume of 75% ethanol was added to the aqueous layer. The RNA was then purified using the RNeasy purification column (Qiagen) according to the manufacturer's protocol. Reverse transcription was performed on 1.5 μg total RNA using the RETROscript kit (Ambion), and quantitative PCR was performed on an MX3000P thermocycler (Stratagene) using Brilliant SYBR Green Master Mix (Stratagene). Primer sequences used were: *Diptericin* (sense, 5′-GCT GCG CAA TCG CTT CTA CT-3′, and antisense, 5′-TGG TGG AGT GGG CTT CAT G-3′), *Drosomycin* (sense, 5′-CGT GAG AAC CTT TTC CAA TAT GAT G-3′, and antisense, 5′-TCC CAG GAC CAC CAG CAT-3′), and control *rp49* (sense, 5′-GAC GCT TCA AGG GAC AGT ATC TG-3′, and antisense, 5′-AAA CGC GGT TCT GCA TGA G-3′).

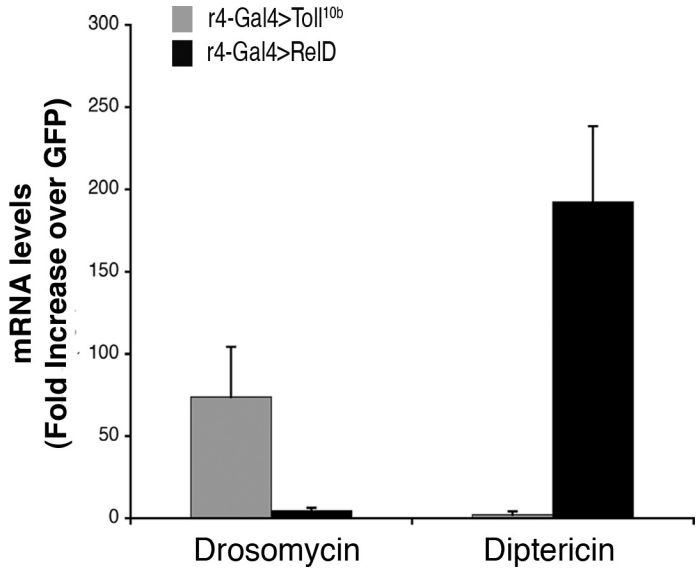

Fig. S1. Activation of the Toll and Imd pathways assessed by induction of *Drosomycin* and *Diptericin* expression. Quantitative PCR was performed on total RNA isolated from *r4-Gal4>Toll*^{10b} and *r4-Gal4>RelD* third instar larvae and compared to *r4-Gal4>GFP* in vial controls. Induction of the antimicrobial peptides *Drosomycin*, a Toll pathway target, and *Diptericin*, an Imd pathway target, is shown as fold increase over *r4-Gal4>GFP* controls. Values are the mean ± SD from at least two experiments.

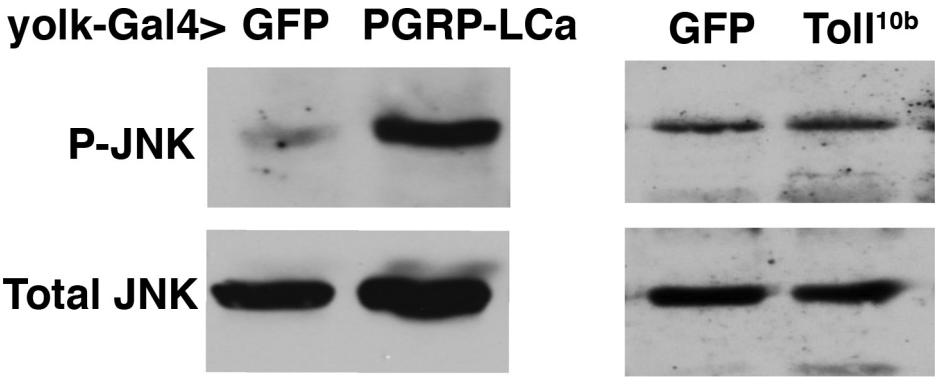

Fig. S2. Immunoblot analysis of phospho-JNK and total JNK from fat bodies isolated from 4- to 5-day-old *yolk-Gal4>PGRP-LCa* and *yolk-Gal4>Toll*^{10b} adult females compared to *yolk-Gal4>GFP* adult female controls generated in the same vial. This experiment was performed three times, and representative blots are shown.

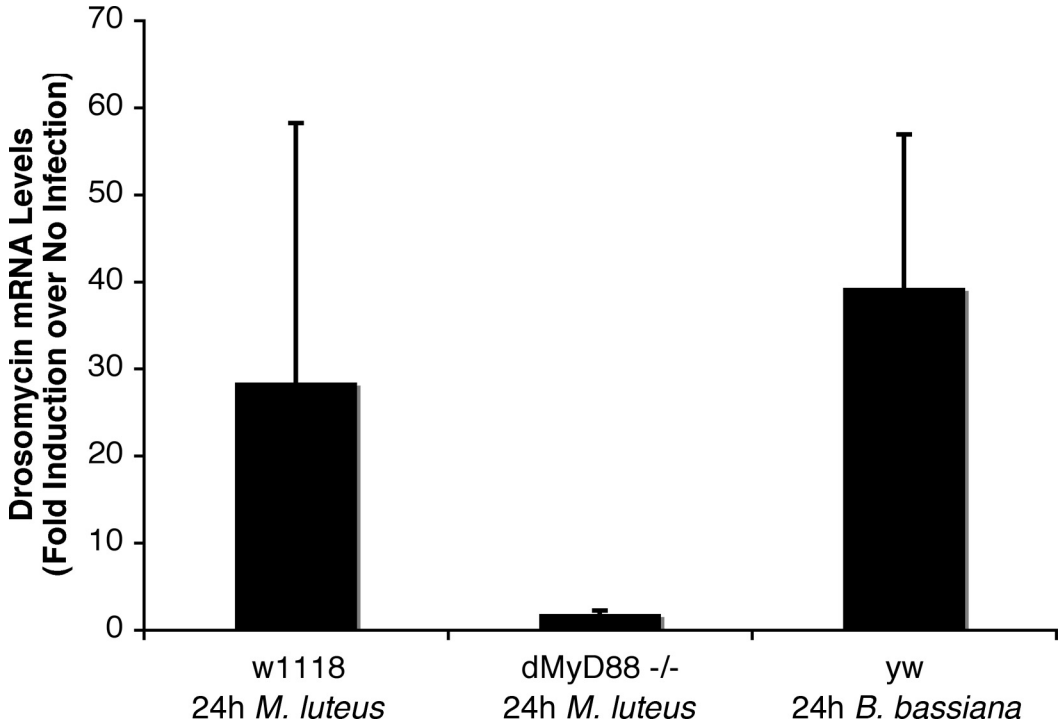

Fig. S3. Activation of the Toll pathway assessed by induction of *Drosomycin* expression. Quantitative PCR was performed on total RNA isolated from 4- to 7-day-old w^{1118} and $dMyD88^{-/-}$ (w^{1118}; $dMyD88^{EP\ (2)2133}$) adult females injected with insulin alone (No infection) or insulin and *M. luteus* (Infection) and *yw* adult females injected with insulin alone (No infection) or insulin and fungal spores (Infection). RNA was isolated from animals 24 h postinfection for *M. luteus* and fungus infection. Induction of the antimicrobial peptide *Drosomycin*, a target of the Toll pathway, is shown as fold increase over No infection controls. Values represent mean ± SD from at least two experiments.

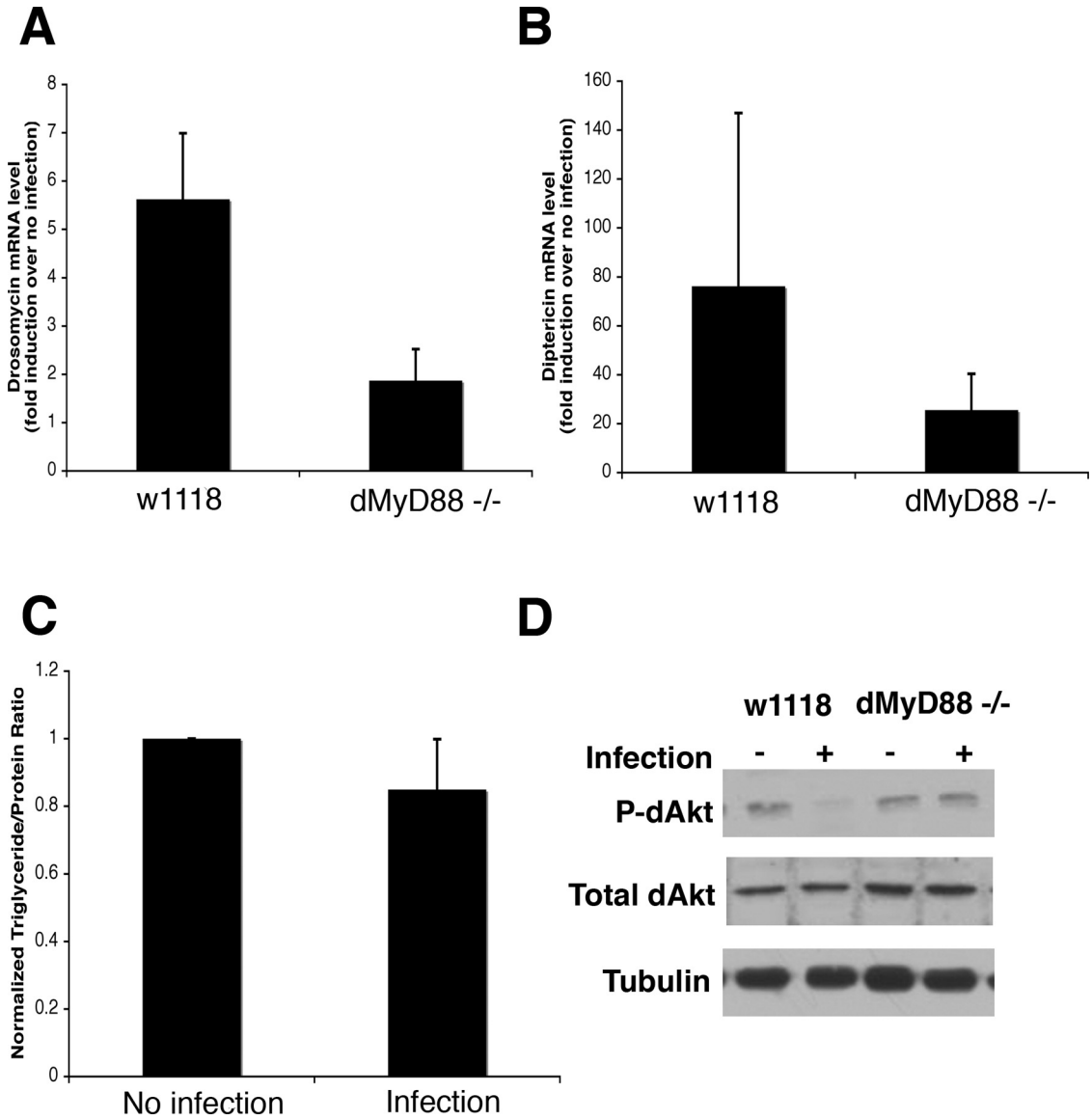

Fig. S4. Infection by *E. coli* causes a Toll-pathway dependent decrease in phospho-dAkt. (*A* and *B*) Quantitative PCR was performed on total RNA isolated from 4- to 7-day-old w^{1118} and $dMyD88^{-/-}$(w^{1118}; $dMyD88^{EP\ (2)2133}$) adult females injected with insulin alone (No infection) or insulin and *E. coli* (Infection). RNA was isolated from animals 10 h postinfection. Induction of the antimicrobial peptide *Drosomycin*, a target of the Toll pathway (*A*), and *Diptericin*, a target of the Imd pathway (*B*), is shown as fold increase over No infection controls. Values represent mean ± SD from at least two experiments. (*C*) Triglyceride/protein ratios from 4- to 7-day-old wild-type (w^{1118}) adult females injected with insulin alone (-) or insulin and *E. coli* (+) 10 h postinfection. This experiment was performed four times and values were normalized to w^{1118} No infection. Values represent mean ± SEM. (*D*) Immunoblot analysis of fat bodies from 4- to 7-day-old wild-type (w^{1118}) and $dMyD88^{-/-}$(w^{1118}; $dMyD88^{EP\ (2)2133}$) adult females injected with insulin alone (−) or insulin and *E. coli* (+) 10 h postinfection. This experiment was performed four times, and representative blots are shown.